

EFFECT OF ZINC SUPPLEMENTATION ON HYPOTHALAMIC– PITUITARY–GONADAL AXIS IN GROWER PIGS

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ABSTRACT

Zinc (Zn) has a wide spectrum of biological activities and its deficiency has been related to various dysfunctions and alterations of normal cell metabolism. The effect of dietary supplementation Zn on Hypothalamic–pituitary–gonadal axis was investigated in the present study. Gilts (24; average body weight 20.65 ± 1.72 kg and age 3 months) were randomly assigned into 3 groups (8 animals / group) receiving a basal diet supplemented with (i) 100 mg/kg of Zn (CON-fed); (ii) 100 mg/kg of Zn along with calcium carbonate (1.5% of dry matter of diet, to induce Zn deficiency) (DEF-fed), or (iii) 500 mg/kg of Zn (FOR-fed) for 120 days of experimental period. Estimation of serum estradiol 17 β (E3) and progesterone (P4) through “COAT-A-COUNT” method of RIA were considered as the marker for activity of hypothalamic–pituitary–gonadal axis of the pigs. Serum E3 level maintain a basal concentration up to day 75 of experiment in all the groups however, in FOR-fed group the concentration of E3 was highest ($P < 0.05$) on day 105 of experiment (age 225 days) compared to CON pigs. The P4 concentration in FOR-fed group was highest on day 105 of experiment. However, the E3 and P4 level was steady in DEF-fed animals up to 120 days of treatment. The higher ($P < 0.05$) values observed in respect of E3 and P4 concentration on day 105 of experiment (at the age of 225) might be due to stimulatory effect of higher dietary Zn level on hypothalamic–pituitary–gonadal axis. The present study suggested that dietary supplementation of Zn at 500 mg/kg level in grower pigs might stimulate Hypothalamic–pituitary–gonadal axis for early attainment of puberty in crossbred pigs.

KEYWORDS: Estradiol 17 β , Grower Pigs, Progesterone, Supplementation, Zinc

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INTRODUCTION

One of the major factors affecting mineral nutrition in pig is the use of cereal products as the main source of diet that is lacking in many of the essential inorganic elements, as well as present in an un-utilizable form in the biological system. These have to be supplemented in biologically available forms to potentiate the desired level of bodily functions to achieve optimum growth and other productive performances. The trace minerals are powerful modulators of several physiological functions that can be considerably perturbed in deficient states (Pathak *et al* 2011.). Apart from controlling the function of nearly 300 biochemical enzymes Zn is considered essential for cell division and synthesis of DNA and protein (Bhowmik *et al*, 2010). For CON-fedling these numerous cellular function it requires a continuous supply along with the diet as Zn is not widely stored in the body (Rink and

Gabriel, 2001). The effect of Zn deficiency on thyroid hormone is well understood (Baltaci *et al*, 2004). Further, our earlier experiment revealed better growth and reproductive performances following dietary supplementation of Zn in grower pigs (Borah *et al*, 2014). However scanty reports are available on the effect of either deficiency or supplementation of Zn on pig hypothalamic–pituitary–gonadal axis. Therefore the experiment was designed to compare the effect of induced Zn deficiency and supplementation of Zn on hypothalamic–pituitary–gonadal axis of gilts through study of serum E3 and P4 level.

MATERIAL AND METHODS

Four months-old (body weight 20.65 ± 1.72 kg), apparently healthy, crossbred (Hampshire \times Assam local) gilts (24), of same genetic group (75% Hampshire) were randomly selected for the experiment. Animals were maintained under uniform feeding, management and housing conditions. The animals were randomly allocated into 3 groups (CON-fed, DEF-fed and FOR-fed), each containing 8 numbers. The basal diet fed to the animals composed of maize (45%), wheat bran (21%), rice polish (10%), de-oiled groundnut cake (10%), soybean meal (7%), fish meal (5%), common salt (0.50%) and mineral mixture (1.5%) during the treatment. The animals were fed twice a day and the amount of feed offered was based upon the body weight with free access to drinking water. All the animals were humanely treated and the experiment was carried out with the due approval of Institute Animal Ethics Committee. The animals were fed the basal diet for a 7 days adaptation period, followed by experimental feeding, namely CON-fed (basal diet supplemented with 100 mg/kg of Zn, treated as CON-fed; DEF-fed (basal diet supplemented with 100 mg/kg of Zn, and calcium carbonate (CaCO₃) @ 1.5% of DM of diet to induce zinc deficiency experimentally); and FOR-fed (basal diet supplemented with 500 mg/kg of Zn). In CON- and DEF-fed groups the Zn supplementation was as per National Research Council (1998) while in FOR-fed group it is based on our hypothesis that whether supplemental Zn has stimulatory effect on hypothalamic–pituitary–gonadal axis of gilts. Serum E3 and P4 was considered as the marker for study of activity of hypothalamic–pituitary–gonadal axis. Venous blood was collected from each of the experimental animals at every 15-days-interval throughout the experimental period and serum was separated. Serum concentration of E3 and P4 were assayed following “COAT-A-COUNT” method of RIA with commercially available kits.

Experimental data were analyzed using ANOVA (Snedecor and Cochran, 1994), followed by *post-hoc* comparison test employing Graph Pad Prism 4.01 software.

RESULTS

The serum E3 concentration in CON-fed, DEF-fed, FOR-fed groups on day of treatment viz day (d) ‘0’ was 25.74 ± 1.51 , 25.13 ± 0.87 , 26.06 ± 1.38 pg/ml respectively (Table 1). The level of E3 was significantly ($P < 0.01$) highest (30.44 ± 1.52 pg/ml) in FOR-fed group on day 90 of the treatment period as compared to the values in CON-fed (25.49 ± 2.1 pg/ml) and DEF-fed group (25.99 ± 0.79 pg/ml). The value of E3 in CON-fed group (31.06 ± 1.87) was significantly ($P < 0.01$) highest on day 120 of the treatment periods as compared to DEF-fed and FOR-fed group.

The serum P4 concentration (Table 1) was significantly ($P < 0.01$) highest in FOR-fed group on d 105 (6.31 ± 0.06 ng/ml) and d 120 (4.2 ± 2.62) of the treatment periods. The CON-fed group showed a significantly ($P < 0.01$) higher value of serum progesterone (3.58 ± 0.04) on day 120 of the treatment periods (Table 1 and 2). The variation in E3 and P4 level in the FOR-fed group was indicative of ovarian stimulation and initiation of follicular wave due to supplementation of zinc at 500 ppm level.

DISCUSSIONS

The E3 concentration on d “0” in all the experimental animals within the range reported earlier (Riskeviciene and Zilinskas, 2001) in gilts. It also revealed that the serum E3 concentration increasing ($P<0.05$) with the approaching age of puberty. The higher ($P<0.05$) level (30.44 ± 1.52) of serum E3 concentration on day 90 of the experiment (pigs attaining age of 210 days; Table 1) is due to attainment of puberty as visual symptoms of estrus was observed in five gilts of this group on evening of day 209 (79th day of experiment) of age.

Higher concentration of E3 in FOR-fed group from day 90 of the treatment period and the concentration of P4 from d 105 reflects the stimulatory effect of supplemented zinc on augmenting the reproductive processes in the peripubertal animals in FOR-fed group. Earlier it was reported that zinc deficiency resulted a lower secretion of GnRH in sheep resulting in delayed in puberty (White 1993). In the present experiment delayed attainment of puberty was recorded in DEF-fed group. This reflects the stimulatory effect of supplemented Zn on GnRH secretion to stimulate the ovarian follicular functions in FOR-fed gilts, as Zn deficiency results in lower secretion of GnRH (Underwood and Suttle, 1999).

The serum P4 concentration was higher ($P < 0.01$) in FOR-fed gilts on d105 (age 225 d) of treatment indicating ovulation, which attained puberty at 218.67 ± 4.51 d. A peak progesterone level was observed in CON-fed gilts on d120 (age 240d), which attained puberty at 237.17 ± 5.27 d. Gilts of DEF-fed group attained puberty at a later age (247.00 ± 1.55 d). Early age at puberty in FOR-fed gilts might be attributed to better growth rate and higher body weight gain as discussed earlier. It might have also influenced follicular development through increased synthesis and secretion of FSH and LH as Zn deficiency in female can lead to impaired secretion of both the hormones resulting ovarian dysfunction and irregular estrous cycle (Bedwal and Bahuguna, 1994). No significant variation was recorded with respect to visual symptoms of estrus viz-duration of estrus and length of estrous cycle among the groups.

Our experiment suggested that higher dose of Zn supplementation (500 mg/kg) might have stimulatory effect on synthesis and secretion of FSH and LH and development of ovarian follicles, which might help in attaining early puberty.

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APPENDICES

Table 1: Serum Estradiol 17-β Concentration (Pg/ML) in Different Experimental Groups during Different Periods of Treatment

Group	Period of Treatment (Age of the Animals in Days)								
	0 (120)	15 (135)	30 (150)	45 (165)	60 (180)	75 (195)	90 (210)	105 (225)	120 (240)
	Serum Progesterone Concentration								
CON-fed	25.74 ± 1.51	25.94 ± 1.20	26.75 ± 1.09	27.01 ± 0.68	26.29 ± 1.49	27.67 ± 1.95	25.49 ^a ± 2.1	25.50 ^a ± 1.97	31.06 ^a ± 1.87
Pre-pubertal	-	-	-	-	-	-	-	-	24.35 (n=2)
pubertal	-	-	-	-	-	-	-	-	*37.12 (n=4)
T ₁	25.13 ± 0.87	25.99 ± 0.87	25.83 ± 1.17	26.89 ± 1.04	25.41 ± 1.09	26.54 ± 0.65	25.99 ^a ± 0.79	25.87 ^b ± 1.05	25.22 ^b ± 1.51
Pre-pubertal	-	-	-	-	-	-	-	-	-
pubertal	-	-	-	-	-	-	-	-	-
T ₂	26.06 ± 1.38	26.51 ± 0.82	26.25 ± 0.32	27.76 ± 0.59	26.27 ± 0.82	27.79 ± 1.58	30.44 ^c ± 1.52	**35.27 ^c ± 7.81	***24.02 ^c ± 7.07
Pre-pubertal	-	-	-	-	-	-	26.12 (n=5)	-	-
pubertal	-	-	-	-	-	-	65.23 (n=1)	(n=6)	(n=6)

Means with different superscript within a column differ significantly.

* Average value of serum progesterone of 2-12 days of cycle.

** Average value of serum progesterone of 3-15 days of cycle.

*** Average value of serum progesterone of 9-20 days of cycle.

Table 2: Serum Progesterone Concentration (NG/ML) in Different Experimental Groups during Different Periods of Treatment

Group	Period of Treatment (Age of the Animals In Days)								
	0 (120)	15 (135)	30 (150)	45 (165)	60 (180)	75 (195)	90 (210)	105 (225)	120 (240)
	Serum Progesterone Concentration (Ng / MI)								
CON-fed	0.24 ±0.02	0.25 ±0.01	0.25 ±0.02	0.26 ±0.01	0.23 ±0.01	0.24 ±0.01	0.23 ±0.04	0.24 ^a ±0.03	3.58 ^a ±0.04
Pre-pubertal	-	-	-	-	-	-	-	-	0.22 (n=2)
pubertal	-	-	-	-	-	-	-	-	*4.2

									(n=4)
T ₁	0.25 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	0.25 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.04	0.23 ^a ± 0.03	0.24 ^b ± 0.04
Pre-pubertal	-	-	-	-	-	-	-	-	-
pubertal	-	-	-	-	-	-	-	-	-
FOR-FED	0.25 ± 0.02	0.25 ± 0.01	0.25 ± 0.02	0.26 ± 0.01	0.24 ± 0.01	0.22 ± 0.02	0.23 ± 0.06	**6.31 ^c ± 0.06	***4.2 ^c ± 2.62
Pre-pubertal	-	-	-	-	-	-	0.26 (n=5)	-	-
pubertal							0.20 (n=1)	(n=6)	(n=6)

Means with different superscript within a column differ significantly.

* Average value of serum progesterone of 2-12 days of cycle.

** Average value of serum progesterone of 3-15 days of cycle.

*** Average value of serum progesterone of 9-20 days of cycle.

